AVENTIS BEHRING GMBH **1999/Z012 - Ma 1216 - C45**

Patent Claims for USA:

1. A process for the preparation in pure form of the protease activating blood clotting factor VII and/or its proenzyme by use of one or more affinity chromatography separation processes and/or fractional precipitation, **which comprises** using, as the affinity chromatography separation process, adsorption on

   - calcium phosphate/hydroxyapatite,
   - a hydrophobic matrix,
   - a chelate matrix,
   - a matrix on which heparin or a substance related to heparin, such as heparan sulfate or dextran sulfate, is immobilized, and/or
   - a matrix which is coated with an immobilized monoclonal or polyclonal antibody directed against the protein to be isolated, or its F(ab) or $F(ab)_2$ fragments.

2. The process as claimed in claim 1, **wherein** one of the chromatographic separation processes or fractional precipitation on its own or each of the abovementioned processes is used in any desired combination with another of the chromatographic separation processes mentioned in claim 1.

3. The process as claimed in claim 1, which is carried out in the presence of one or more protein stabilizers which are selected from the groups consisting of

- complexing agents of divalent ions, preferably EDTA, EGTA or citrate, and/or
- divalent ions, preferably calcium ions, and/or
- amino acids, preferably glutamate, arginine, lysine or glycine, and/or
- sugars, preferably glucose, arabinose, mannose or mannitol, and/or
- solubilizers, preferably hydroxyproline, and/or
- detergents, preferably Tween® or Triton®, and/or
- alcohols, preferably ethylene glycol or polyethylene glycol, and/or
- proteins, preferably albumin, gelatin, fibronectin, vitronectin or similar proteins, and/or
- reductants, preferably dithiothreitol, mercaptoethanol or cysteine, and/or
- proteinase inhibitors such as aprotinin, α-2-antiplasmin, C1-esterase inhibitor, the inter-α-trypsin inhibitor, the antithrombin III/heparin inhibitor or synthetic inhibitors.

4. The process as claimed in claim 1, **wherein**, for the affinity chromatography separation of the protease activating factor VII from its proenzyme by means of stepwise elution, a substance which has bonds of different strength to the protease on the one hand and to the proenzyme on the other hand is

immobilized on the support material, the different eluates are then collected separately from one another and the respective protein is isolated from them.

5. The process as claimed in claim 1, **wherein** the fractional precipitation of the protease and/or its proenzyme from its solution is carried out by addition of

   - polyethylene glycol from a concentration of at least 10% by weight or

   - ammonium sulfate from a concentration of at least 15% by weight.

6. A pharmaceutical preparation, **which comprises** the protease activating blood clotting factor VII and/or its proenzyme together with one or more protein stabilizers as set forth in claim 3, for assisting blood clotting in the case of a tendency to bleeding, in the case of a lack of factors of the endogenous clotting pathway, as FEIBA or for the prophylaxis and/or therapy of syndromes associated with thrombotic complications, in inherited or acquired deficiency states of the protease or its proenzyme, for assisting wound healing alone or as a constituent of a fibrin adhesive, a web and in combination with growth factors for subcutaneous, intramuscular, intravenous or topical treatment.

7. The use of a pharmaceutical preparation of claim 6 for the coating of surfaces of articles consisting of plastic or metals to be implanted in the body, such as synthetic heart valves, blood vessels or cannulas inserted for taking blood or for artificial feeding.

8. A reagent comprising the protease activating blood clotting factor VII and/or its proenzyme together with one or more protein stabilizers as set forth in claim 3 for use in biological test systems and for antigen detection.